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(54) Title: TOPICAL OPHTHALMIC PREPARATIONS CONTAINING IMMUNOSUPPRESSIVE AGENTS

(57) Abstract

Therapeutic preparations for topical ophthalmic application, containing from 0.02 to 5.0 weight % of immunosuppressive agents belonging to the groups of monocyclic undecapeptides, macrolide lactones or corticosteroids, in a vehicle comprising up to 10 weight % of polyalkyleneglycol–polyurethane copolymers. Said copolymers consist preferably of poly(oxy–1,2–ethanediyl)–α–hydro–ω–hydroxypolymers with 1,1'–methylene–bis–(4–isocyanatocyclohexane) having an average molecular weight of from 1000 to 3000 in a hydrophilic vehicle and preferably of poly[oxy(methyl–1,2–ethanediyl)]–α–hydro–ω–hydroxypolymers with 1,1'–methylene–bis–(4–isocyanatocyclohexane) having an average molecular weight of from 1600 to 18000 in a lipophilic vehicle. Said therapeutic agents can further contain additional excipients common in topical administration forms.

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# TOPICAL OPHTHALMIC PREPARATIONS CONTAINING IMMUNOSUPPRESSIVE AGENTS

## Technical Field

The invention relates to therapeutic preparations destined for topical ophthalmic application of lipophilic immunosuppressive agents, dissolved or dispersed in physiologically acceptable vehicles comprising excipients which enhance penetration of therapeutic agents into ocular tissues.

## Background Art

Topical ophthalmic application includes both external administration of therapeutic preparations into the conjunctival sac under the lid and onto the cornea and subconjunctival and retrobulbar administration by injection.

The efficiency of topically administered pharmaceuticals for the treatment of ocular diseases is influenced, besides of anatomical and physiological characteristics of the eye, especially by physical and chemical characteristics of the therapeutic agent and by the character of the administration form and its composition. It is well known that the transport of therapeutic agents into ocular tissues is hampered by defensive mechanisms of the eye such as increased lacrimation and blinking, which decrease the concentration of the therapeutic agent in the precorneal area. Absorption of the therapeutic agent is enabled by prolonged contact with the cornea and by the penetrating capacity of the therapeutic agent. The bioavailability of the therapeutic agents following topical administration onto the eye is usually as low as 1 to 10 % of the agent administered.

Typical lipophilic immunosuppressive agents which can be used for the treatment of ocular diseases include for example agents having anti-inflammatory and immunosuppressive activity belonging to the groups of monocyclic poly-N-methylated undecapeptides, of lactonic macrolides or of corticosteroids. Undecapeptides suitable for ophthalmic treatment comprise the group of cyclosporins, particularly ciclosporin, $[Nva]^2$-ciclosporin, $[Val]^2$-ciclosporin and dihydro$[Val]^2$-ciclosporin.

Macrolide antibiotics, suitable for the treatment of ocular diseases, include especially tacrolimus and sirolimus. Tacrolimus and structurally similar derivatives are generally designated as ascomycins; sirolimus and structurally similar derivatives are generally designated as rapamycins.

The group of corticosteroids useful in topical ophthalmic application includes substances like, for example, cortisone and hydrocortisone, prednisolone and methyl prednisolone, dexamethasone and fluoromethalone.

Therapeutic effects of the above cyclosporins in topical administration are demonstrated especially in risky transplantations of the cornea and in autoimmune diseases such as keratoconjunctivitis sicca, chronic keratitis, Behcet's syndrome, Sjögren's syndrome, endogenous uveitis, but also in ulcerative diseases of the cornea such as Moren's ulcus.

The macrolide antibiotics have proved their high immunosuppressive effects in organ and tissue grafting and are promising in keratoplasty. The corticosteroids are useful in therapy of inflammations following ocular surgery and they also suppress immune reactions following cornea grafting.

All the tree groups of the active agents described in this invention have also been administered in a systemic way for the treatment of inflammatory and autoimmune diseases of the eye. However, systemic administration can only be used to a reduced extent because of serious side effects of such therapy. The side effects associated with systemic administration can be reduced or eliminated in rational topical administration by concentrating the activity of the preparation only to the site treated and its nearest neighbourhood.

The therapeutic agents from the groups of N-methylated cyclic undecapeptides, macrolide lactones and corticosteroids in their non-ionised form have only a low solubility in water or in hydrophilic carriers which are physiologically acceptable for ophthalmic administration. That, in fact, makes preparation of classical hydrophilic eye drops very difficult. For example, water solubilities of the cyclosporins range between 16 and 30 μg/ml at 25 °C, the more polar molecule of $[Thr]^2$-ciclosporin being more water soluble than the less polar $[NVa]^2$-ciclosporin. For relative evaluation of lipophilicity of the agents from said groups, the values of partition coefficient P between the aqueous phase and the lipophilic phase represented by n-octanol can be used. For corticosteroidal agents, for example, the value of log P ranges from 1.2 (prednisolone) to 4.3 (flurbiprofene). In the group of

cyclosporins, log P for agents having immunosuppressive activity ranges from 1.1 to 3.1. No values of partitions coefficients for ascomycins and rapamycins have been published, but it can be assumed based on water solubilities that they will be comparable to those for cyclosporins.

The first patents disclosing ophthalmic topical administration of agents from the group of cyclosporins as a method of treatment of phaco-anaphylactic endophthalmitis and uveitis in the anterior and posterior ocular segments or of eye diseases manifested by reduced lacrimal production include US patents 4,649,047 and 4,839,342. Both these patents do not closely specify the administration form and both envisage use of solutions, suspensions and ointments with a pharmaceutically acceptable vehicles comprising vegetable, animal, mineral and silicone oils; liposomes and, further, alcohols, dimethyl sulfoxide and polyoxyethylated castor oil. Said patents disclose no weight ratios of suitable excipients in the examples or in the claims.

US patent 4,865,846 relates to the group of corticosteroids, cyclosporins and antibiotics for ophthalmic administration and it claims transport systems in which solutions of therapeutic agents in a liquid or ointment base are present together with particles of biodegradable materials containing the same or even different therapeutic agents as well as a method of preparing such transport systems. It concludes from the examples of said patent that preparing of three-dimensional particles having a size of from 0.4 to 1.0 mm from bioerodible materials such as collagen, gelatine, polyvinylalcohol and methylcellulose derivatives, suspending said particles in a liquid or ointment base as well as incorporating therapeutic agents into said bioerodible particles is very laborious and not very suitable for a validable production process.

Topical preparations for administration to the eye and surrounding tissues containing cyclosporins in a vehicle composed of a mixture of vegetable oils and vaseline are disclosed in UK patent 2,224,205. The therapeutic agents obtained by the methods described in this patent, however, contain, as emulsifiers, substances based on steroidal materials from sheep wool. A departure from using such materials has been reported recently for possible allergenetic and irritative effects of possible insecticidal residues included in the wool fat.

Ophthalmic preparations having low concentrations of cyclosporin in an aqueous medium containing surfactants from the group of polyethoxylated fatty acid esters, polyethoxylated alkyl ethers and polyethoxylated alkyl phenyl ethers are described in the

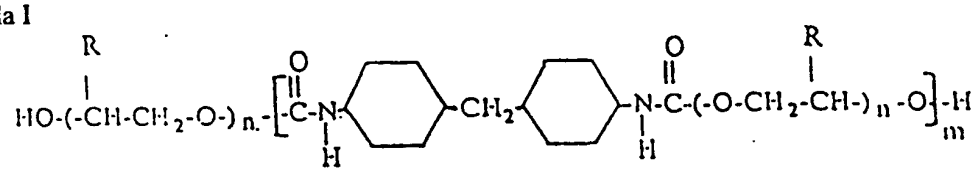
international patent application WO 93/23010. Emulsified ophthalmic formulations having specific affinity to tear glands, containing cyclosporins, are described in the international patent application WO 95/31211.

Euacidic and isotonic nanoemulsions useful for application, containing from 0.01 to 5 % of therapeutic agents including corticoids and cyclosporins, are described in the European patent application EP 0 696 452 A1. None of the above mentioned patent documents solves the specific problem of increasing penetration of the therapeutic agent through the cornea into the internal ocular structures such as the aqueous humour, iris or uvea.

## Disclosure of the Invention

The object of the present invention is to increase penetration through the cornea of topically administered therapeutic agents, thus obtaining therapeutically active levels of the therapeutic agents in the internal tissues of the eye, by means of use of especially suitable adjuvants in the therapeutic agents. This task is extremely difficult in the case of therapeutic agents which are very poorly soluble in body fluids and tissues. Generally, therapeutic agents which are very poorly soluble in water are those substances which require 1000 to 10000 volume parts of water to dissolve 1 weight part of the substance. Such agents include especially immunosuppressive agents from the group of monocyclic undecapeptides represented by ciclosporin or $[Nva]^2$-ciclosporin, from the group of lactones having macrolide structures represented by sirolimus or tacrolimus, and from the group of corticoids represented for example by prednisolone or dexamethasone.

The present invention describes therapeutic preparations for topical ophthalmic application, which are characterised by the presence of from 0.02 to 5.0 weight % of immunosuppressive agents, dissolved or dispersed in physiologically acceptable vehicles comprising up to 10 weight % of polyalkyleneglycol-polyurethane copolymers of general formula I



(I),

wherein n is between 8 and 51, m is predominantly between 1 and 4 and R means $CH_3$ or H.

The chemically correct CAS names of these compounds are given in the shortened CTFA nomenclature throughout the description below:

| CAS nomenclature | CTFA nomenclature |
|---|---|
| Poly (oxy 1,2-ethanediyl)-α-hydro-ω-hydroxy polymer with 1,1'-methylene-bis-(4-isocyanatocyclohexane) CAS No. 39444-87-6 | PEG-8/SMDI copolymer |
| Poly[oxy (methyl 1,2-ethanediyl)-α-hydro-ω-hydroxy polymer with 1,1'-methylene-bis-(4-isocyanatocyclohexane) CAS No. 9042-82-4 | PPG-12/SMDI copolymer |
| CAS No. 9042-82-4 | PPG-51/SMDI copolymer |

In the CTFA nomenclature, PEG and PPG correspond to polyethyleneglycol and polypropyleneglycol, resp., and SMDI corresponds to saturated methylene diphenyl isocyanate.

Said compounds are marketed, for example, under trade designations Polyolprepolymer-2 (PPG-12/SMDI copolymer), Polyolprepolymer-14 (PPG-51/SMDI copolymer) and Polyolprepolymer-15 (PEG-8/SMDI copolymer) and are produced by PENEDERM Incorporated.

In dermal use, these copolymers have been known. They have a strong activity to the skin and easily form deposits in the stratum corneum. Thus, long-persistent liquid reservoirs of therapeutic agents are formed among the corneocytes, which enable prolonged action of the therapeutic agents in the skin.

Use of polyol prepolymers in dermal and cosmetic preparation is claimed, for example, in US patents 4,971,800, 5,045,317 and 5,051,260.

In spite of considerable differences in the anatomic structure of the skin from that of the ocular cornea, it has been surprisingly found that polyalkyleneglycol-polyurethane copolymers markedly increase penetration of very poorly soluble immunosuppressive agents through the cornea. Said phenomena has been demonstrated in ophthalmic preparations

wherein the above described therapeutic agents were incorporated in the presence of said copolymers into both hydrophilic and hydrophobic, physiologically harmless vehicles.

It has also been surprisingly found that the therapeutic agents of the invention having a content of polyalkyleneglycol-polyurethane copolymer excipients in the applicable concentration range up to 10 weight % do not produce any increase of ocular irritability, although demonstrably increased penetration of therapeutically active immunosuppressive agents into internal ocular structures occurs. Moreover, histological examination following repeated administration of the preparations according of the invention does not indicate any possibility of damage thereof. On the contrary, manifestations of ocular irritability and toxicity have been described for the administration azone in concentrations which are as low as 0.1 % and higher; azone being a known substance used for enhancing permeability of therapeutic agents through the cornea (Ismail I.M. et al., Pharm. Res., 9(6), 817-821, 1992).

Obtaining good biological characteristics is dependent on qualitative parameters of the copolymers of this invention, especially on residual concentrations of the unreacted diisocyanate component and the respective polyalkylene glycols. Typical parameters of the copolymers are summarised as follows:

| | **PPG-12/SMDI copolymer** | **PPG-51/SMDI copolymer** | **PEG-8/SMDI copolymer** |
|---|---|---|---|
| **mol. weight (weight average)** | 4000 | 18000 | 1800 |
| **viscosity mPa.s at 37 °C** | 2500-4500 | 2500-6000 | 2500-5000 |
| **unreacted diisocyanate** | max. 0.1 % | max. 0.1 % | max. 0.1 % |
| **residual**<br>**PPG content**<br>**PEG content** | max. 20 %<br>- | max. 20 %<br>- | -<br>max. 20 % |
| **heavy metals content** | max. 20 ppm | max. 20 ppm | max. 20 ppm |
| **water content** | max. 0.1 % | max. 0.1 % | max. 0.1 % |

The polyalkylene-polyurethane copolymers comprise mixtures of oligomers the proportion of which is expressed by the index „m“ in the general formula, amounting predominantly to from 1 to 4.

Various types of the copolymers produced have typical molecular weight distributions as determined by gel permeation chromatography. For example, for the PPG-12/SMDI copolymer, the proportions of the molecular weights are:

from 22 to 23 % (for the molecular weight 1500-2000 peak)
from 16 to 25 % (for the molecular weight 2400-3300 peak)
from 28 to 45 % (for the molecular weight ≥ 3700 peak)

Both types of copolymers having terminal hydroxyl groups in their polyalkyleneglycol parts markedly increase deposit of the mentioned therapeutic agent into the middle part of the cornea (stroma), which has a high content of water. Due to stepwise lipophilicity of the copolymer fractions having different molecular weight a concentration gradient forms in the cornea, which is the cause why the very poorly water-soluble immunosuppressive agents are not fixed only in the strongly lipophilic layer of the corneal epithelium formed by only approximately 5 upper layers of living cells.

Lipophilic ophthalmic vehicles consist of, on one hand, vegetable or animal triacyl glycerols (e.g., castor oil, maize oil, sesame oil or fish oil) and hydrogenated forms thereof. Hydrocarbon mixtures such as saturated hydrocarbons hexamethyl tetracosaene known as vaseline or unsaturated branched hydrocarbons known as squalene, or the hydrogenated form thereof - squalane - can also be incorporated among triacyl glycerols in limited amounts. Lipophilic vehicles also include structured lipids consisting of triglyceryl esters having medium long $C_8$ to $C_{12}$ acyl residues in combinations with long $C_{14}$ to $C_{22}$ acyl residues. Also polyglyceryl esters of $C_{14}$ to $C_{22}$ fatty acids having a value of hydrophilic-lipophilic balance (HLB) lower than 6 and having an oily character are useful as non-irritating lipophilic ophthalmic vehicles. Also the $C_{12}$ to $C_{23}$ fatty alcohols can be used for this purpose.

Hydrophilic ophthalmic vehicles for the preparations of the invention generally consist of water or alkylene glycols such as, particularly, glycerol or polyethylene glycols. Also useful are ethyleneoxide-propyleneoxide block copolymers, known as poloxamers or meroxapols.

Both types of ophthalmic vehicles are also characteristic in having a liquid or semi-solid consistency at temperatures of from 15 to 37 °C.

Additional excipients may be present in the vehicles of the topical ophthalmic preparations according to this invention in technologically necessary amounts and the choice thereof is dependent on the specific administration form selected. For solutions, such

excipients include solvents and solubilising materials, physiologically acceptable for the eye, having a liquid or semi-solid consistency. In emulsifiable and suspendable dispersion preparations, such excipients additionally include stabilisers of the present phases. In multi-dose packings of the ophthalmic preparations, anti-microbial agents are usually added.

As the molecular weight of the PEG-8/SMDI copolymer does not exceed 3000, it will be advisable to take its contribution to the osmotic pressure in the preparations with a hydrophilic vehicle into account and, if needed, to adjust the tonicity in a usual way with additional excipients having a ionic or non-ionic character.

## Brief description of the drawings

The appended drawings are graphic illustrations of the effects of the addition of 1 % of the PPG-51/SMDI copolymer to topical ophthalmic preparations of Example 3 on absorption of ciclosporin into the cornea (diagram 1) and its penetration into the iris (diagram 2), into the aqueous humour (diagram 3) and into the uvea (diagram 4). A significant increase in absorption occurred in the groups of pigmented male and female rabbits. The average increase of the ciclosporin concentration following 12 administrations of 1 drop each was as follows: cornea, 3.30 x; iris, 5.24 x; aqueous humour, 5.08 x; and uvea, 5.2 x.

Table I depicting the scheme of the evaluation of the ocular reaction is also appended.

## Examples

The following examples describe various compositions of the preparation of this invention without being limiting in any way.

*Example 1 Eye drops* *1%* *2%*

| | | |
|---|---|---|
| Ciclosporin | 1.000 kg | 2.000 kg |
| PPG-12/SMDI copolymer | 1.00 kg | 1.00 kg |
| Diglyceryl monooleate | 2.50 kg | 5.00 kg |
| Chlorobutanol | 0.50 kg | 0.50 kg |
| Maize oil | up to 100.00 litres | up to 100.00 litres |

In a kettle of 100 l volume equipped with a Steridose Systems AB magnetic stirrer PPG-12/SMDI copolymer and diglyceryl monooleate are mixed with maize oil. Freshly sublimed chlorobutanol and ciclosporin are dissolved in the resulting mixture at ambient temperature. The solution is aseptically filtered into a sterilised reservoir through a membrane filter having the separation limit of 0.2 μm. The product is filled into polyethylene vials having the volume of 5 ml on a BFS system apparatus.

Both concentration variants of the preparation were evaluated for ocular irritability in rabbits according to the method described below.

Eye drops of Example 1 containing 1 % and 2 % ciclosporin and the corresponding placebos without the copolymer were evaluated for ocular irritability by the method recommended by the ETAD committee for toxicology.

Eye drops were tested each on 6 New Zealand white rabbits. The animals were of a conventional quality having weights of from 2.19 to 4.62 kg, corresponding to their ages. The rabbits were housed individually in metal cages and acclimatised to the vivarium conditions (temperature of 22 ± 2 °C, relative humidity of 50 to 70 %). The rabbits were fed with standard KKK/L diet and drinking water ad libitum.

Eye drops were administered in a single dose of 50 μl into each eye, divided into two sequential portions of 25 μl each. The condition of the eye (conjunctiva, cornea, iris and tear production) was monitored before administering and 24, 48 and 72 hours after administering. The cornea was examined using commercially available fluorescein solution at a blue point light. The physiological solution was applied to the eyes in the same manner and the same amount after 24 hours. Changes in the conjunctiva, cornea, iris and tear production were evaluated according to the scheme given in Table I. Cumulative values of the changes detected after 24, 48 and 72 hours in all the 6 rabbits are the following:

| | 1% Eye drops | | 2% Eye drops | |
|---|---|---|---|---|
| | Preparation | Placebo | Preparation | Placebo |
| **Changes in cornea** [A x B x 5] | 0 | 0 | 0 | 0 |
| **Changes in iris** [A x 5] | 0 | 0 | 0 | 0 |
| **Changes in conjunctiva** [A x b x C] x 2 | 32 | 10 | 16 | 0 |
| **Resulting degree of irritability** | 1.78 | 0.56 | 0.89 | 0 |
| **Final evaluation** | non-irritating | non-irritating | non-irritating | non-irritating |

$$\text{Resulting degree of irritability} = \frac{\Sigma \text{ changes in cornea, iris and conjunctiva}}{[3 \times 6]}$$

Classification of irritability:

| | | |
|---|---|---|
| resulting degree | 0 to 10 | non-irritating |
| | 11 to 25 | slightly irritating |
| | 26 to 56 | moderately irritating |
| | 57 to 110 | strongly irritating |

| ***Example 2 Eye drops*** | ***A*** | ***B (placebo)*** |
|---|---|---|
| **Ciclosporin** | 2.000 g | 2.000 g |
| **PPG-51/SMDI copolymer** | 2.000 g | - |
| **Decaglyceryl pentaoleate** | 5.000 g | 5.000 g |
| **Tocopheryl linoleate** | 0.700 g | 0.700 g |
| **Maize oil** | up to 100.000 ml | up to 100.000 ml |

The preparations were evaluated for tolerance in administration in dogs for 19 days according to the method described with histopathologic examination and penetration of ciclosporin into ocular tissues was evaluated.

10 male Beagle dogs aged 12 to 16 months were used for the test. The dogs were treated in periodic intervals. In the appropriate period they were treated with Canvac, Dohyvac-Parvo and Lyscelin vaccines.

The animals were housed individually in cots having dimensions 0.9 x 1.0 m under conventional conditions. They were fed with standard pelletised Ro XIII diet at a ration of 300 g for each dog. Drinking water was available to the dogs ad libitum.

The tested preparations according to Example 2 were administered four times daily to 5 dogs (test group) in two-hours intervals one drop onto the cornea of each eye by means of a dropper from the standard packing. After dropping, spreading of the preparation on the cornea was achieved by slight clamping of the eyelids. A control group (5 dogs) was administered placebo in the same manner. Both preparations were administered for 19 sequential days. Ophthalmologic examinations were made before the start of the administration and at the end of the administration period. Ocular tolerance was again evaluated according to the scheme in Table I, daily before the first administration. Presence and extent of possible cloudiness of the cornea (degrees from 0 to 4), condition and reaction of the iris (degrees from 0 to 2), presence of erythema or oedema of the conjunctivas (degrees 0 to 4) and presence of secretion (degrees 0 to 3) were evaluated. Both variants A and B were classified as non-irritating.

Two dogs were use for histopathologic examination, which were administered 1 drop of the preparation of Example 2 into each conjunctival sac four times daily. A control dog was also tested, which was administered placebo in the same manner. The duration of the test was 19 days.

1 hour after the last administration the dogs were killed by bleeding in thiopental anaesthetisation. Eyeballs were carefully removed from the orbits and fixed in 10% neutral formol. Using a sharp razor blade, an incision was made through each eye, extending from the entry of the ocular nerve to the edge of the cornea. The larger ocular segment was then used to make a lamella of a width of about 8 mm, which contained the cornea, pupil, lens and optic nerve. The respective tear glands were removed from the outer part of each orbit close to the eyeball.

The histological preparations were made by standard paraffin method, cut in a thickness of about 6 μm and stained with hematoxylline-eosine, by the PAS method for

mucopolysaccharides, with cresyl violet, with Alcian blue, by the method of Van Gieson and by the Halle-Müller technique.

Both the histotopographs of the eyeballs of the control dog and those of both dogs which were administered the preparation of Example 2 did not show any anomalies in any of the recorded ocular structures (cornea, sclera, conjunctiva, chorioid, retina, ciliary body, iris, lens, anterior and posterior chambers, optic nerve, sections of eye muscles and peribulbar tissue). Neither the conjunctiva lining the posterior area of the upper eyelid, neither the tear gland were changed.

Penetration into eye tissues

Groups of 4 Beagle dogs aged 12 to 16 moths each were used in the study. Three of them were administered, to both eyes, preparation A or B according to Example 2, specifically labelled with tritiated ciclosporin having an activity of 2 mCi/ml in an amount of 0.03 ml. Administrations were made 4x daily in two-hour intervals for two days; on the third day the preparation was administered three times. A total of 11 administrations were made. The fourth dog received the preparation of Example 2 without specific labelling in the same amount and frequency.

One hour following the last administration the dogs were killed by bleeding from carotids in thiopental anaesthetisation. Both eyes were then removed from each dog, including conjunctivae and tear glands. Right eyes from all the four dogs were embedded in 2% carboxymethylcellulose gel and frozen in a n-hexane/dry ice mixture for autoradiographic assay. Samples of 25 % of the individual tissues were taken from left eyes, weighed and then dissolved in 1 ml 25% KOH in 20% ethanol. Radioactivity was measured using a Wallac Rackbeta liquid scintillation spectrophotometer. The activity values measured (dpm) were calculated as ng of the substance administered/g tissue.

| | A | | B | |
|---|---|---|---|---|
| | Ave. conc. (n = 3) | Stand. dev. | Ave. conc. (n = 3) | Stand. dev. |
| **Cornea** | 15743.4 | ± 2730.2 | 5100.2 | ± 1474.1 |
| **Conjunctiva** | 4350.2 | ± 192.3 | 750.0 | ± 97.7 |
| **Iris** | 633.5 | ± 62.2 | 120.9 | ± 44.5 |
| **Tear gland** | 146.6 | ± 44.1 | 30.8 | ± 23.0 |
| **Aqueous humour** | 62.0 | ± 7.9 | 11.7 | ± 3.4 |

The measured values of ciclosporin in the vitreous body, lens, sclera and retina were at the background level.

*Example 3 Eye drops*

| | *A* | *B* |
|---|---|---|
| **Ciclosporin** | 2.00 g | 2.00 g |
| **PPG-51/SMDI copolymer** | 1.00 g | - |
| **Diglyceryl monooleate** | 4.00 g | 5.00 g |
| **Maize oil** | up to 100.00 ml | up to 100.00 ml |

Both formulations were labelled with tritiated ciclosporin in the amount of 1 mBq/mg of the active substance. Both preparations were administered to a group of 6 rabbits weighing 3 to 3.5 kg each, consisting of 3 males (M) and 3 females (F). Preparation A was instilled into the left eye, always onto the cornea (not into the conjunctival sac).

Administrations were made at 15 minute intervals for 3 hours (a total of 12 administrations) at an amount of 15 µl of the preparation by means of a micropipette (a total of 3.6 mg ciclosporin). 15 minutes following the last administration the animals were killed, aqueous humours were taken from all eyes and the cornea, iris and uvea were prepared.

The tissue samples were weighed and treated for radioactivity measurements at a Wallac Rackbeta liquid scintillation spectrohoptometer. The measured activity values (dpm) were calculated as concentration of ng ciclosporin/g tissue.

The average values of tissue concentrations of ciclosporin (n = 6) in both preparations are the following:

| | **A** | **B** |
|---|---|---|
| **Cornea** | 14246.1 ng/g | 5832.1 ng/g |
| **Iris** | 915.9 ng/g | 174.8 ng/g |
| **Aqueous humour** | 293.1 ng/g | 57.8 ng/g |
| **Uvea** | 107.4 ng/g | 20.3 ng/g |

*Example 4 Eye ointment*

| | |
|---|---|
| **$[Nva]^2$ - ciclosporin** | 0.50 g |
| **PPG-51/SMDI copolymer** | 2.00 g |
| **RRR-α-tocopherol** | 0.40 g |
| **Hydrogenated castor oil** | 26.9 g (CUTINA® HR from HENKEL) |
| **Castor oil** | 70.00 g |

Tocopherol. the copolymer and the active substance are dissolved in the sterilised mixture of castor oil and hydrogenated oil at 60 °C, hot filtered under aseptic conditions and filled into tubes fitted with ocular applicators.

*Example 5* *Eye drops*

| | |
|---|---|
| **Sirolimus** | 0.50 g |
| **PPG-12/SMDI copolymer** | 1.00 g |
| **Sorbitane trioleate** | 2.00 g |
| **Ascorbyl palmitate** | 0.02 g |
| **Soybean oil** | up to 100.00 ml |

The prepared solution is filtered through a membrane having the separation limit of 0.2 µm and is filled into glass vials fitted with ocular applicators.

*Example 6* *Eye drops*

| | |
|---|---|
| **Dexamethasone** | 0.100 g |
| **PEG-8/SDMI copolymer** | 6.000 g |
| **PEG-50 stearate** | 5.000 g (Myrj® 53 from ICI) |
| **$K_2HPO_4$** | 0.061 g |
| **NaOH 0,1 M solution** | q.s. pH 6.9 |
| **Phenylethyl alcohol** | 0.300 g |
| **Sterilised water** | up to 100.00 ml |

The prepared solution is filtered through a membrane having the separation limit of 0.2 µm and is filled into polyethylene vials fitted with ocular applicators.

Industrial applicability

The invention can find application in pharmaceutical industry in producing topical ophthalmic preparations. The preparations of the invention are characterised in high penetration of immunosuppressive agents into ocular tissues and in a very good tolerability.

Table I

## Evaluation scheme of eye reaction

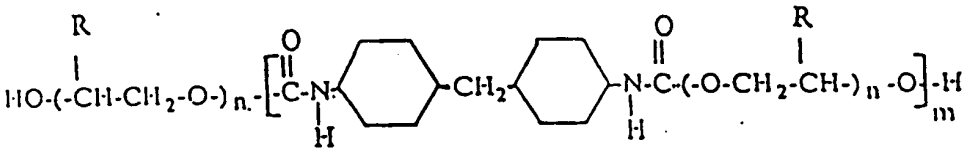
| Cornea [calculation of changes in the cornea A x B x 5; max. value 80] | | |
|---|---|---|
| *degree* | *A Cloudiness* | *B Area of changes in the cornea* |
| 0 | No cloudiness | No area |
| 1 | Disseminated or dispersed area cloudiness, iris details clearly visible | One quarter and less (but not none) |
| 2 | Easily distinguishable areas of cloudiness, iris details slightly unclear | More than a quarter to a half |
| 3 | Opalescent areas, non-distinguishable iris details, pupil magnitude hardly | More than a half to three quarters |
| 4 | Cloudy surface, iris not clear | More than three quarters to whole area |

| Conjunctiva [calculation of changes in the conjunctiva ( A+B+C) x 2 ; max. value 20] | | | |
|---|---|---|---|
| *degree* | *A Erythema of lids and eyes* | *B Oedema* | *C Lacrimation* |
| 0 | No erythema | No swelling | Physiological tear formation |
| 1 | Slightly injected vessels of conjunctival sac | Slight oedema (including nictitating membrane) | Slightly increased lacrimation (not including slight increase of tear formation observed in the angle) |
| 2 | Diffuse erythema of conjunctiva, individual vessels hardly distinguishable | Clear swelling with partial detachment of lid | Lacrimation with lid and lash moistening |
| 3 | Diffuse dark erythema | Swelling with palpebral phimosis to approx. one half | Lacrimation with lid, lash and eye neighbourhood moistening |
| 4 | | Swelling with palpebral phimosis to approx. one half or lids are fully shut | |

| Iris [calculation of changes in iris A x 5; max. value 10] | |
|---|---|
| *degree* | *A Evaluation of changes* |
| 0 | Physiological condition of iris |
| 1 | Ciliation without changes, adhesion, swelling; injected vessels (all the mentioned changes, or some of them) iris still reacts to changes |
| 2 | Haemorrhage, large destruction, iris does not react to light (all the mentioned changes, or some of them) |

Claims:

1. Therapeutic preparations for topical ophthalmic application, characterised by the presence of from 0.02 to 5.0 weight % of immunosuppressive agents, dissolved or dispersed in a physiologically acceptable vehicle comprising up to 10 weight % of polyalkyleneglycol-polyurethane copolymers of general formula I



(I),

wherein n is between 8 and 51, m is predominantly between 1 and 4 and R means the group $CH_3$ or hydrogen,

optionally together with additional excipients common for topical administration forms.

2. Preparations of claim 1 characterised in that in case of a hydrophilic vehicle the polyalkyleneglycol-polyurethane copolymers are poly(oxy-1,2-ethanediyl)-α-hydro-ω-hydroxypolymers with 1,1'-methylene-bis-(4-isocyanatocyclohexane) having an average molecular weight of from 1000 to 3000.

3. Preparations of claim 1 characterised in that in case of a lipophilic vehicle the polyalkyleneglycol-polyurethane copolymers are poly[oxy(methyl-1,2-ethanediyl)]-α-hydro-ω-hydroxypolymers with 1,1'-methylene-bis-(4-isocyanatocyclohexane) having an average molecular weight of from 1600 to 18000.

4. Preparations of claim 1 to 3 characterised in that the immunosuppressive agents are selected from the groups of monocyclic undecapeptides or macrolide lactones or corticosteroids and are used either individually or in any mutual mixtures.

5. Preparations of claim 1 to 4 characterised in that the immunosuppressive agents are ciclosporin and/or $[Nva]^2$-ciclosporin in an amount of from 0.1 to 3.0 weight % and/or tacrolimus and/or sirolimus in an amount of from 0.05 to 2.0 weight % and/or dexamethasone and/or prednisolone in an amount of from 0.02 to 1.0 weight %.

Diagram

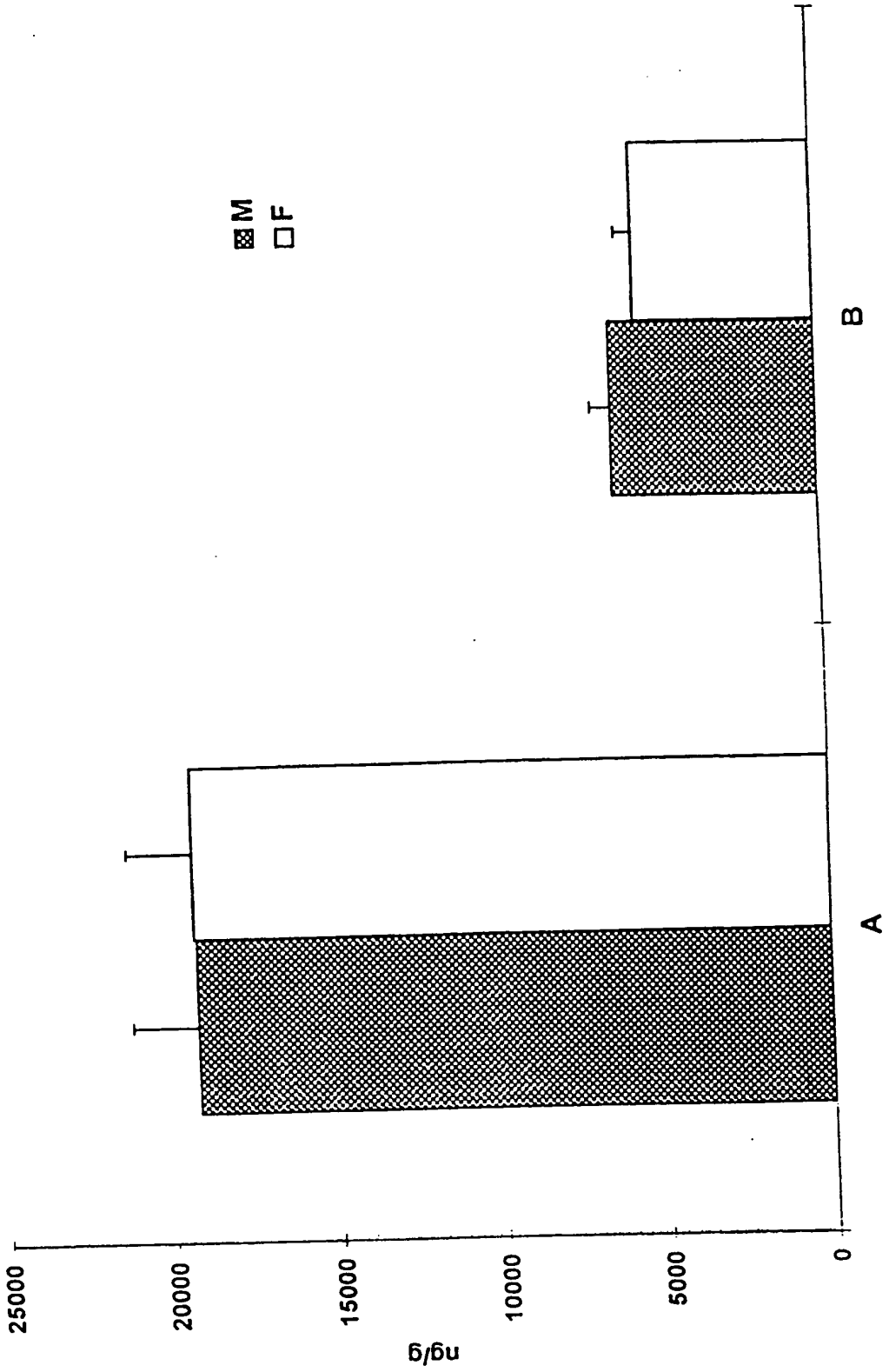


Diagram 2

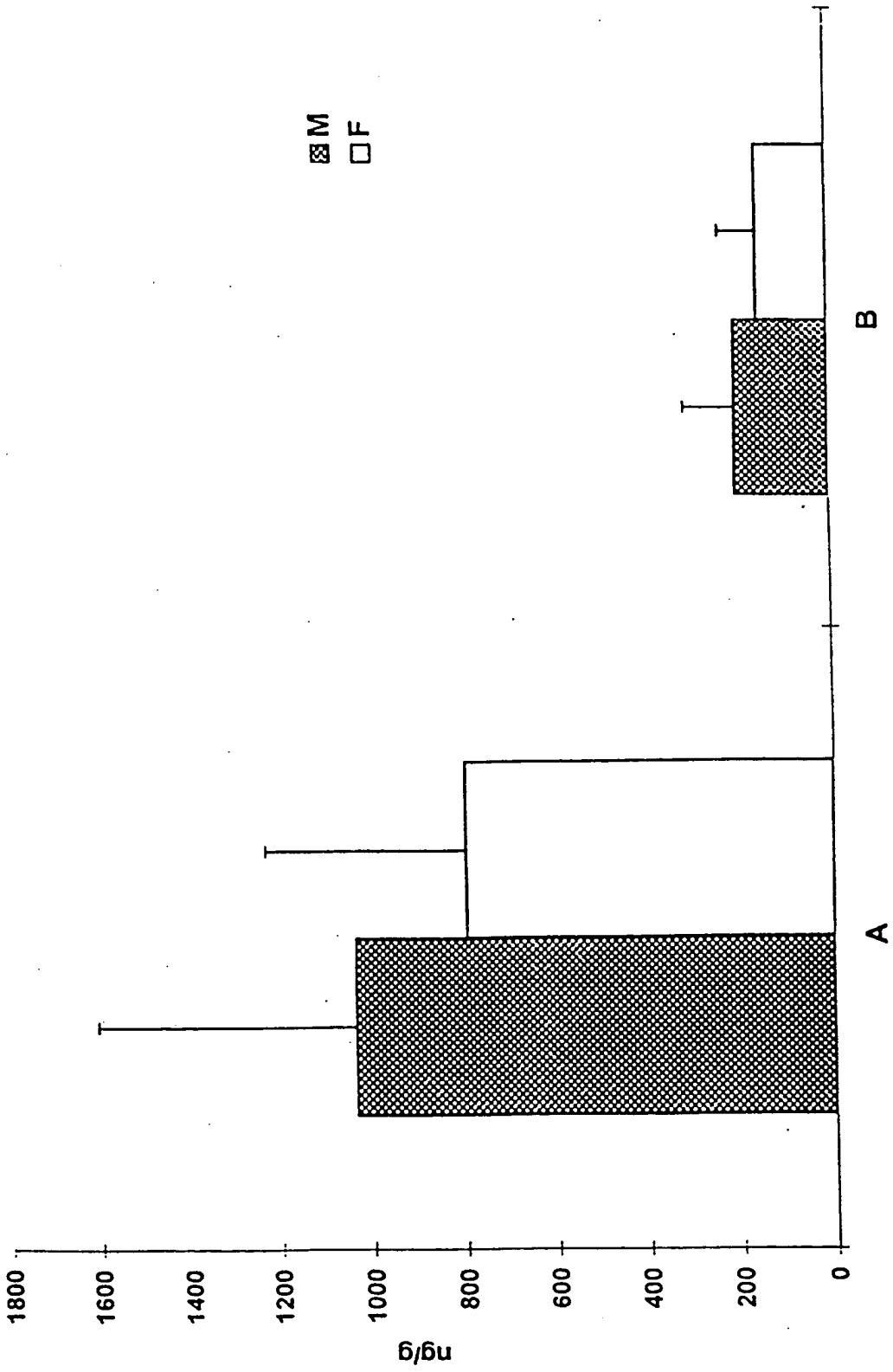


Diagram 3

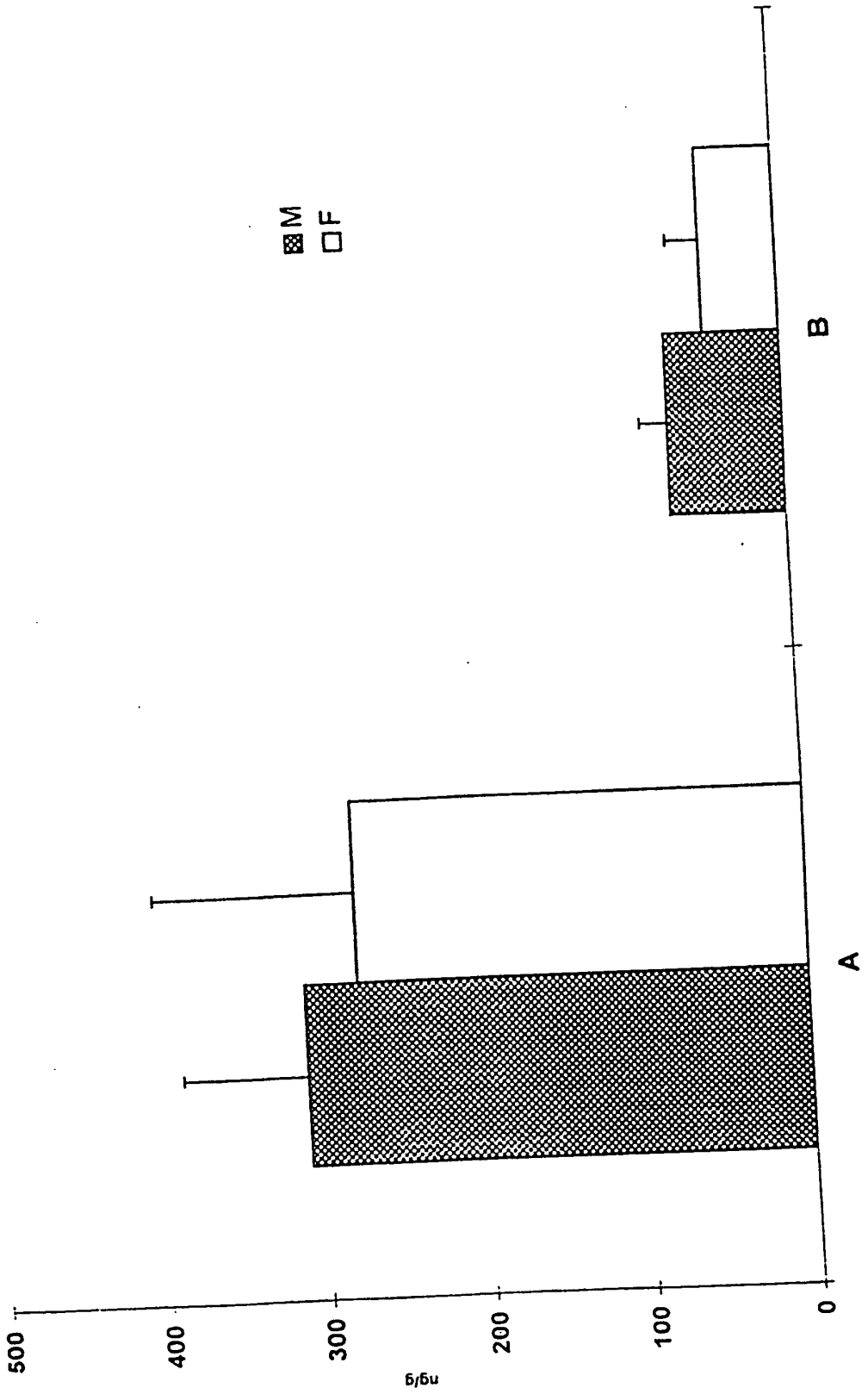
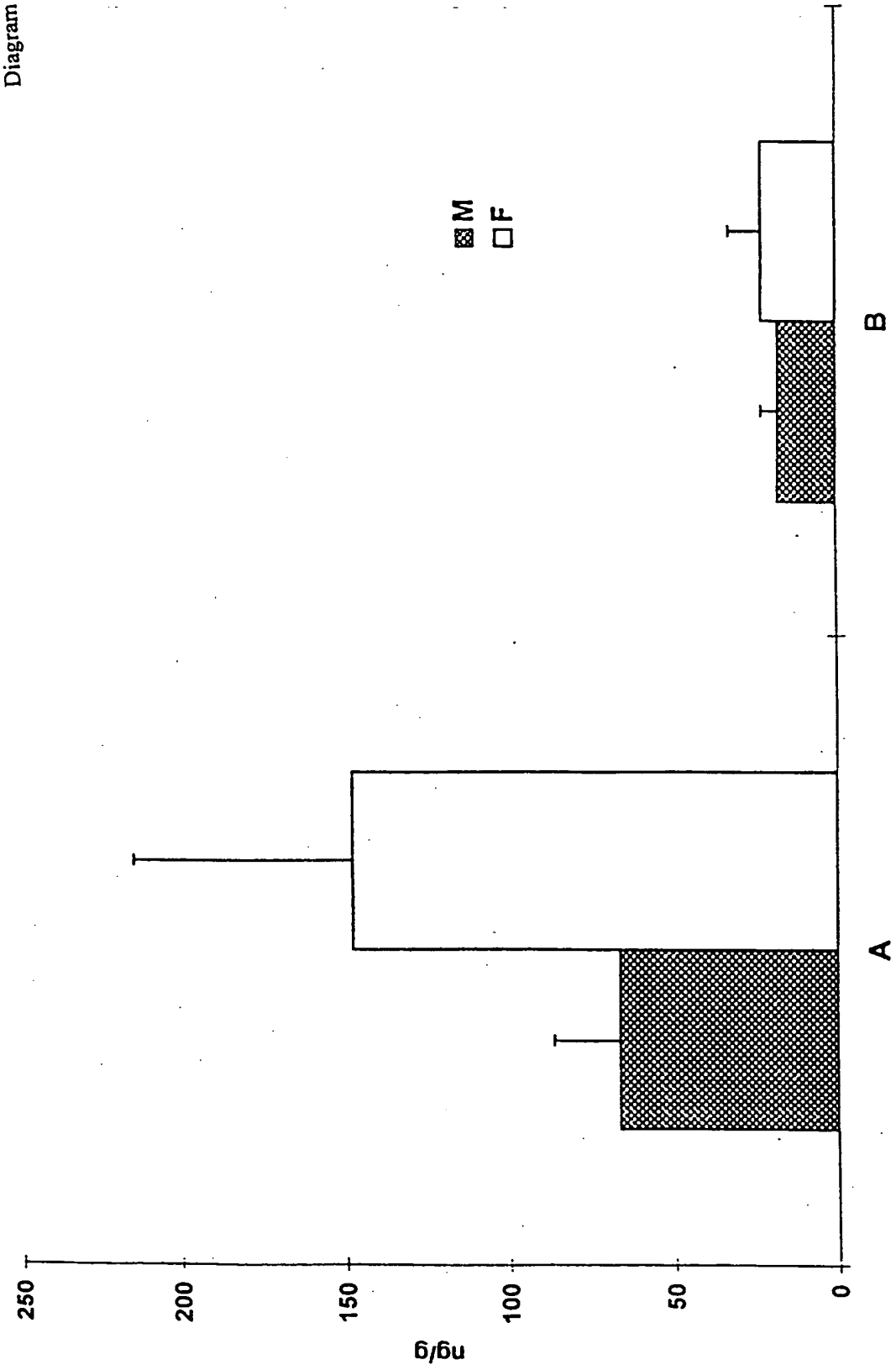


Diagram 4



# INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/CZ 98/00054

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| Y | --- | 1-6 |
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| Y | --- | 1-6 |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 4 March 1999 | 12/03/1999 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Seegert, K |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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| Y | ----- | 1-6 |

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Information on patent family members

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